A Half-Century of Studies on a Chromosomal Hybrid Zone of the House Mouse

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Abstract

The first natural chromosomal variation in the house mouse was described nearly 50 years ago in Val Poschiavo on the Swiss side of the Swiss-Italian border in the Central Eastern Alps. Studies have extended into neighboring Valtellina, and the house mice of the Poschiavo-Valtellina area have been subject to detailed analysis, reviewed here. The maximum extent of this area is 70 km, yet it has four metacentric races and the standard 40-chromosome telocentric race distributed in a patchwork fashion. The metacentric races are characterized by highly reduced diploid numbers (2n = 22 – 26) resulting from Robertsonian fusions, perhaps modified by whole-arm reciprocal translocations. The races hybridize and the whole Poschiavo-Valtellina area can be considered a ‘hybrid zone’. The studies of this area have provided insights into origin of races within hybrid zones, gene flow within hybrid zones and the possibility of speciation in hybrid zones. This provides a case study of how chromosomal rearrangements may impact the genetic structure of populations and their diversification.

Introduction

The house mouse *Mus musculus* has been domesticated to become one of the standard models in biology and medicine: the laboratory mouse (Phifer-Rixey and Nachman 2015). Because of the wealth of genetic, and now genomic, information on the house mouse, its natural populations also form the mammalian evolutionary model (Boursot et al. 1993; Sage et al. 1993; Macholán et al. 2012; Phifer-Rixey and Nachman 2015). For instance, the only known 'speciation gene' in mammals was discovered in mice (Mihola et al. 2009), the best-studied mammalian hybrid zone is that between *M. m. domesticus* (the western house mouse) and *M. m. musculus* (the eastern house mouse)(Baird and Macholán 2012), the genetics behind phenotypic traits are being elucidated (Gray et al. 2015), as are fundamental population genetic processes (Didion et al. 2016). That the house mouse normally has a chromosome complement of 40 telocentric chromosomes has been known since the early 1900s, but only within the last 50 years has it been discovered that *M. m. domesticus* has a variable karyotype (Garagna et al. 2014). Given this karyotypic differentiation and the status of the house mouse as an evolutionary model, the species has also become a primary model for the study of chromosomal variation.

The first indication of chromosomal variability in the house mouse came from Val Poschiavo in Switzerland, on the border with Italy. Here, in 1969, Alfred Gropp discovered a 26-chromosome largely-metacentric population of mice (Gropp et al. 1969). Val Poschiavo is a narrow valley on the south-facing side of the Alps, isolated from the rest of Switzerland by a high mountain pass, but forming a side valley of the much wider Valtellina in Italy (Figure 1). The human residents of these two converging valleys not only share geography, but have also had linguistic, social and agricultural ties for centuries (Hauffe et al. 2004). At the time of Gropp et al.'s paper, the Poschiavo mice were already well-known for their unusual black-and-tan coloration and classified as a separate species (and named as such in the paper: the 'tabakmaus' or tobacco mouse *Mus poschiavinus*, for their habit of colonizing the once numerous tobacco-curing kilns). This chromosomally distinctive population of mice is now known as the Poschiavo metacentric race of *M. m. domesticus* (abbreviated as CHPO, according to the standard nomenclature: Piálek et al. 2005) rather than as a separate species, following the awareness that coat color variation is common (from sandy grey to almost black), and not sufficient grounds for species designation (Hauffe et al. 2004). Subsequent to Gropp et al.'s original study, it was shown that the mouse populations from Valtellina also vary in chromosomes, coat color and other genetic markers (Hauffe et al. 2004). Because there are several chromosomal races in addition to CHPO that meet and interbreed in the area, it can be classified as a 'chromosomal hybrid zone' (Hauffe et al. 2012). Therefore, the whole Poschiavo-Valtellina area (Figure 1) is fascinating from the viewpoint of mouse evolutionary genetics, but here we will focus on the properties of the chromosomal races and the nature of their interactions.

The Poschiavo-Valtellina area is not the only area of chromosomal variation in the house mouse. Chromosomal variation within *M. musculus* is almost entirely limited to the *domesticus* (western) subspecies, but has been described in various regions, including parts of North Africa, the Near East and western Europe and several Atlantic islands (Piálek et al. 2005). In all these regions chromosomal races of house mouse differ from the standard 40-chromosome telocentric karyotype by the presence of sets of metacentric chromosomes, similar to the CHPO race. The metacentric condition and reduced diploid number reflects occurrence of Robertsonian (Rb) fusion mutations (fusion of non-homologous telocentrics at their centromeres), although the actual metacentrics that are present in a particular chromosomal race can also be the result of whole-arm reciprocal translocations (WARTs; the swapping of chromosome arms between two metacentrics or a metacentric and a telocentric)(Figure 2; Searle

1993). Metacentric races have diploid numbers varying between 2n = 38 and 2n = 22 (or 1 to 9 pairs of metacentrics) with the even chromosome numbers reflecting the homozygous condition. Thus, different metacentric races are defined by different sets of autosomal metacentrics. However, it is important to note that the variation is not limited to variation in *number* of metacentrics: the *arm combinations* of those metacentrics also varies. Of a total of 171 possible combinations, 101 have been described in the (coincidentally) 101 metacentric races reviewed by Hauffe et al. (2012). Only the sex chromosomes have not been found as components of metacentrics in the chromosomal races of house mice described thus far. In the Poschiavo-Valtellina area the following ten metacentrics have been described (α.β, where α and β represent the chromosome arms of the metacentric, homologous to unattached telocentrics): 1.3, 2.8, 4.6, 5.15, 7.18, 8.12, 9.14, 10.12, 11.13 and 16.17 (Table 1).

With such a variety of metacentric races, there are numerous chromosomal hybrid zones wherever those metacentric races meet the standard telocentric race (ST40), and where metacentric races come into contact with each other (Hauffe et al. 2012). The Poschiavo-Valtellina hybrid zone area involves different pairwise contacts in close proximity (Figure 1) involving four different metacentric races (see Figure 3 for examples of karyotypes) and ST40 (Table 1). Pattern and process are beautifully intermingled in this system. The variety of different races reflects colonization and evolutionary processes, and having this variety of races spatially dispersed in a relatively small geographical area leads to fascinating interactions. In many ways the Poschiavo-Valtellina hybrid zone area is a dynamic microcosm of evolutionary process involving chromosomal variation in the house mouse. In this article we will consider how this hybrid zone area is a model system in multiple respects: for studying the origin of new chromosomal races, for studying the interaction of chromosomal races and for studying speciation resulting from chromosomal rearrangements.

Distribution of Mice and Races

The Adda and Poschiavino Rivers, which flow through the Poschiavo-Valtellina area, are flanked in all directions by the Central Eastern Alps, with valley bottoms at altitudes ranging from 200 – 1200 m (highest village in Val Poschiavo: Angeli Custodi, 1200 m; highest village in Valtellina: Sommacologna: 1000 m; Figure 1), except to the west where it is delimited by Lake Como. The house mice that have been studied chromosomally are from discrete villages distributed along the valley floor of the Adda (over the 70 km between Morbegno and Sondalo), and the Poschiavino (20 km between Tirano and Angeli Custodi in Val Poschiavo), with gaps between villages ranging from several hundred meters to a few kilometers, on both sides of the rivers (Figure 1).

Although in this region there are seasonal movements of people and livestock between lower and higher altitudes, house mice are largely confined to the permanently inhabited villages of the valley floor. Whenever we have tried live-trapping rodents in summer habitations in the higher altitude alpine meadows (even those just above the villages), we have not caught house mice, but only field mice of the genus *Apodemus* (HC Hauffe, personal observations), presumably for climatic reasons and the very seasonal nature of food resources. Likewise, house mice are absent in natural and agricultural habitat between villages on the valley floor (HC Hauffe, personal observations), where again they are presumably out-competed by other murids; i.e. house mice in the Poschiavo-Valtellina area are highly commensal (Cucchi et al. 2005), and totally dependent on humans (‘anthrodependants’: Hulme-Beaman et al. 2016). We believe there is the opportunity for occasional voluntary long-distance movement between these villages (mark-recaptured house mice have been proven to travel over 1 km; Pocock et al. 2005), but mouse movement between villages is more likely to be by involuntary transport of mouse-infested agricultural supplies and produce, such as straw, hay or animal feed. These movements presumably occur most frequently between neighboring villages but could also be from very long distances, much further than would be possible by a self-propelled mouse. As well as inhospitable habitats between villages, the Adda and Poschiavino Rivers are likely strong barriers to mouse movement, since although they are not very wide, they are cold and fast-flowing. In addition, until recently, very few bridges spanned these torrents (Hauffe et al. 2004).

The genetic characteristics of the house mice in the villages will therefore reflect the colonization event(s) that seeded the population in the village, plus later influx of individuals through movement of produce and occasional self-propelled movement. Many of the villages are dominated by a single race (Hauffe and Searle 1993; Hauffe et al. 2004; Figure 1), suggesting that initial colonization is important in defining the chromosomal characteristics of a village population, but the frequent presence of chromosomally-defined hybrids indicates subsequent immigration events (Figure 1). There is a certain degree of order in terms of the distribution of chromosomal races, i.e. the Poschiavo race (CHPO) dominates Val Poschiavo (blue-shaded area in Figure 1), and the Lower Valtellina race (ILVA), Lower Valtellina (yellow-shaded areas in Figure 1); the Mid Valtellina race (IMVA) is more prevalent on the north side of the Adda River in the upper reaches of Valtellina (villages 4-7, Figure 1), and the Upper Valtellina race (IUVA) dominates the south side of the Adda River in Upper Valtellina (villages 13-15), and both sides in the middle section of this valley (villages 16-22; Figure 1, Table 1). However, there are isolates of CHPO and ST40 within this distribution and, as implied earlier, many villages appear to have mice with chromosome complements reflecting hybridization with mice of neighboring villages (e.g. villages 4 and 16, Figure 1). In the wider context of previously described hybrid zones, this zone shows a remarkable patchwork of races, with each patch at most only several hundred meters or a few kilometers in length (Figure 1), whereas the norm in hybrid zones is a linear contact between two races (Barton and Hewitt 1985). Searle (1993) labelled this a 'mottled' hybrid zone to distinguish it from 'mosaic' hybrid zones described by Harrison and Rand (1989), where the patchiness reflects different habitat associations, exemplified by the cricket *Gryllus*, where the hybridizing forms prefer different soil types.

With regards the origins of the patchiness of chromosomal characteristics in house mice in the Poschiavo-Valtellina area, such a distribution is inevitable, since the species is restricted to a single habitat type (due to its anthrodependence), and the villages themselves have a discontinuous distribution with five races (Table 1) in a small area, with single villages or groups of neighboring villages being dominated by a particular race. Clearly the precise distribution of the races reflects the vagaries of colonization history, and in addition, both genetic drift (Polechová and Barton 2011) and selection (Piálek et al. 2001) may have influenced which race predominated in situations where multiple metacentric races simultaneously colonized the same village. Another factor believed to have enhanced the patchiness in the Poschiavo-Valtellina area is village-level extinction, followed by subsequent recolonization from a distance, leading to the population exhibiting different chromosomal characteristics from those of neighboring villages. These various factors contributing to the patchiness described for the Poschiavo-Valtellina area are completely different from the *Gryllus*-type of mosaic hybrid zone, where two hybridizing forms clearly associate with and are adapted to different habitats, and the patchy distribution of the forms reflects the patchy distribution of the habitat (Larson et al.

2013). There is no evidence to suggest that the different chromosomal types in the house mouse are better adapted to one village rather than another.

The particular historical circumstances that likely led to village-level extinctions and recolonization in this area are interesting to consider. Most of the mice that have been karyotyped in the Poschiavo-Valtellina area belong to a metacentric race (Hauffe et al. 2004; Figure 1). However, in Vervio (village 9), Nova (village 10), Tovo di S. Agata (village 11) and Mazzo (village 12) in Upper Valtellina, the dominant karyotype is the telocentric ST40; this karyotype is presumably also found in Lower Valtellina given the hybrids found in Berbenno (village 24; Figure 1). The telocentric-dominated Upper Valtellina villages represent a part of the valley that was submerged by a flood for at least a year in 1807, following a huge landslide opposite Sernio (village 17) that blocked the Adda River causing a lake to form that extended from below Sernio to Grosotto. Hauffe and Searle (1993) suggested that the mouse colonization following human reoccupation of the villages may have been from afar, rather than from neighboring villages (characterized by mice with metacentric karyotypes). Villages recovering from such a devastating flood would bring in new supplies from various sources, and it would appear a prime opportunity for a very long distance colonization event. The presence of ST40 in Lower Valtellina (Figure 1) may also represent a very long distance colonization following local extinction. Not only is the Poschiavo-Valtellina area dominated by metacentric races, so are neighboring valleys (Piálek et al. 2005; Burt et al. 2009); therefore, ST40 mice in the area may have been brought in with supplies deriving from hundreds of kilometers away as the nearest known ST40 populations outside of Valtellina are in Val Chiavenna (Burt et al. 2009), near Milan (Gropp et al. 1982; Piálek et al. 2005), and near Lake Garda (Hauffe et al. 2011; HC Hauffe and JB Searle unpublished data).

Model of raciation

To understand the racial diversity of the Poschiavo-Valtellina area, it is important first to put it in a much wider context. The metacentric races in the Poschiavo-Valtellina area represent four out of 15 races in the North Italian System (NIS) of metacentric races that extends across the southern Italian Alps to the Po River, flanked by the Maggiore and Garda Lakes, and the Ticino and Mincio Rivers (Piálek et al. 2005; Burt et al. 2009).

The metacentric that defines all the races in this system is metacentric 16.17, although other metacentric chromosomes are also widely shared. There are many additional metacentric races north of the Swiss-Italian border (forming the Northern Europe–Northern Switzerland and the Southern Switzerland Systems; Piálek et al. 2005), such that there is a large continuous area where house mice are characterized by metacentric chromosomes, extending from northern Italy through eastern Switzerland and western Austria to southern Germany (Piálek et al. 2005). It is likely that this region of metacentric races (the largest such metacentric-dominated area in the house mouse) has a single origin because, although the races north of the Swiss-Italian border are distinctive from the NIS and are in different Systems, there is still some sharing of metacentrics, e.g. metacentric 9.14 is widespread (though not ubiquitous). This metacentric is particularly dominant in the NIS, including the Poschiavo-Valtellina area.

Thus, from a putative single origin, a huge number of metacentric races have arisen (55; over half those recorded in the house mouse: Piálek et al. 2005; Hauffe et al. 2012), leading to the large metacentric-dominated area in and around the Alps. Given the presumed ancestral condition of an all telocentric karyotype (Garagna et al. 2014), Rb fusions would have been needed, because this is the only type of whole-arm rearrangement that can create new metacentrics from such an ancestor (Figure 2). Indeed, a single origin of the metacentric races in the region would suggest that there was initially a metacentric race homozygous for a single metacentric. Modification of this first metacentric race would have involved sequential addition and change of metacentrics, in a setting of population expansions and contractions, following a more complex version of the classical model of sequential formation of chromosomal races by White (1978). New chromosomal races could have originated with or without geographical isolation, and at different times, and would have expanded to different extents. The most derived chromosomal races would, of course, have originated most recently and could occupy a very small area. As well as Rb fusions being necessary for the origin of the metacentric condition in central Europe, the diversity of chromosomal races can also partially be explained by Rb fusions, if the fusions are added stepwise to the karyotype and if there is expansion of races after each mutation. This can be followed by subdivision and further addition of metacentrics, with several cycles of this process and different metacentrics added to the separate subdivided populations; WARTs can add further diversity. When karyotypes have a fully metacentric autosomal complement (2n = 22), WARTs are the only way to instantaneously generate entirely new metacentrics. Given the loss of centromeric DNA upon fusion of two telocentrics (Garagna et al. 1995, 2001), it is thought that it is not easy for Rb fissions to occur (Figure 2), disrupting a possible pathway to generate new metacentrics from a largely metacentric karyotype. Considering the Poschiavo-Valtellina area, it has been proposed specifically that the metacentrics within the four metacentric races have originated by Rb fusions and WARTs (Piálek et al. 2005). The involvement of WARTs is very likely because permitting this type of mutation has been shown to substantially reduce the number of evolutionary steps in chromosomal phylogenies over those that only allow Rb fusions (Hauffe and Piálek 1997; Britton-Davidian et al. 2005; White et al. 2010). Pairs of neighboring races elsewhere within the NIS are also particularly convincingly related by WARTs (Garagna et al. 1997; Hauffe and Piálek 1997).

However, the diversity of metacentric races is not limited to fixation of de novo mutations; new races may arise through the generation of recombinant forms following hybridization, with the recombinant product having a combination of metacentrics derived from the parental races. This formation of new chromosomal races occurs within hybrid zones, and has been termed 'zonal raciation' (Searle 1993; White et al. 2010). Again, zonal raciation can reduce the number of evolutionary steps in chromosomal phylogenies (Hauffe and Piálek 1997; White et al. 2010).

In the Poschiavo-Valtellina area we have recently found the most direct evidence for zonal raciation in the house mouse or any other mammal (confirming a suggestion by Hauffe and Searle in 1993). Within the Poschiavo-Valtellina area the distribution of races (Figure 1) suggests that CHPO and ILVA are ancestral (these ancestral races are defined by the following metacentrics: CHPO: 8.12; ILVA: 2.8, 10.12, 7.18; Table 1). These two races mainly occupy Val Poschiavo and Lower Valtellina respectively and presumably arose in allopatry. The other two races, IMVA and IUVA, could have arisen by recombination from the hybridization of CHPO and ILVA (the recombinant races are defined by the following metacentrics: IMVA: 8.12, 7.18; IUVA: 2.8, 10.12; Table 1). The F_1 hybrid between the CHPO and ILVA produces two meiotic multivalents (Figure 4) and is expected to suffer substantial infertility from germ cell death and malsegregation of chromosomes leading to aneuploidy and embryonic death, although not complete sterility (Searle 1993; Hauffe and Searle 1998). Under these circumstances, a recombinant race may have been favored because of a better reproductive performance than either parental race; this is because a recombinant homozygote interbreeding with either parental race can only produce a hybrid with a single multivalent which will not suffer the same extreme unfitness as the hybrid between the parental races. Simulation modelling shows that recombinant homozygotes can go to fixation because of this selective advantage (Piálek et al. 2001), which could explain the high frequency of one recombinant north of the Adda River in Upper Valtellina (IMVA) and the other recombinant to the south of the river (IUVA) (Figure 1).

Both for the study of raciation and for the gene flow studies in the next section, we have made use of the fact that CHPO and ILVA built up genome-wide genetic differences, presumably because they were isolated in allopatry before coming into contact and hybridizing. Although hybridization erodes these differences (as examined in the next section), by focusing on the loci most closely linked to chromosomal rearrangements that define the CHPO and ILVA races, we are able to track the history of those rearrangements following an approach originally used by Riginos and Nachman (1999). Those most closely linked loci are positioned at the centromeres of the rearrangements. Despite this hybridization, we hypothesized, as Riginos and Nachman had done, that centromeric loci on race-specific chromosomes would continue to remain differentiated. We were able to show that the centromeric microsatellite genotypes of chromosomes 10 and 12 were similar for the races CHPO and IMVA and distinctive from races ILVA and IUVA which were themselves similar to each other (Giménez et al. 2016). Likewise, for chromosomes 7 and 18, the centromeric microsatellite genotypes were similar for the races CHPO and IUVA and distinctive from races ILVA and IMVA which were themselves similar to each other (Giménez et al. 2016). These results are precisely what is expected if IMVA and IUVA are recombinant products produced by zonal raciation. In addition, the mice from San Giacomo, a village in Lower Valtellina which has both mice of ILVA (one of the ancestral races) and IUVA (one of the recombinant races) and hybrids between them (village 22; Figures 1 and 3), shows perfect segregation of alleles at a centromeric microsatellite locus for chromosome 7 among these races. The locus concerned is D7Mit306 with six ILVA homozygotes having a genotype 109/109, eight ILVA x IUVA F_1 hybrids having a genotype 101/109 and ten IUVA homozygotes having a genotype 101/101 or 101/107. This case illustrates that there are markers that are stably inherited and associate with different rearrangements of the same chromosome and therefore appropriate for these studies of hybrid race formation.

Together, these studies have suggested that race formation in the Poschiavo-Valtellina area has involved formation of metacentrics by Rb fusions and (almost certainly) WARTs, and shuffling of those metacentrics by zonal raciation to generate the four metacentric races that we now see there.

Model of a hybrid zone

A chromosomal hybrid zone is characterized by the presence of hybrids between chromosomal races and the hybrids include heterozygotes for chromosomal rearrangements. Arising from the properties of these heterozygotes, gene flow between hybridizing chromosomal races may be expected to occur less readily close to rearrangement breakpoints than elsewhere in the genome (Panithanarak et al. 2004). Firstly, recombination suppression around the breakpoints of the rearranged chromosomes may be expected in chromosomal heterozygotes, reducing gene exchange in these regions (Rieseberg 2001; Ortiz-Barrientos et al. 2016). Secondly, chromosomal heterozygotes may be expected to show reduced fitness due to meiotic aberrations (Searle 1993). In this case the rearrangement breakpoint acts as an unfitness locus, and the impact in terms of reduced gene flow between hybridizing races will be greatest closest to that locus because the probability of recombination between the unfitness locus and another locus will be lower the closer the second locus is to the unfitness locus (Panithanarak et al. 2004; see Barton and Hewitt 1981).

The importance of reduced gene flow in certain chromosomal regions is two-fold. Considering the races making contact and hybridizing, a restriction to gene flow maintains and potentially allows the build-up of genetic differences between the races. Thus, the races can be considered 'evolutionarily significant units' (Moritz 1994). They are not separate species, but they are still a unit of diversity, and a contribution to biodiversity; distinct genetic entities that represent an interesting and important partition of genetic diversity within a species. Of course, genetically distinct races that are not exchanging genes freely may potentially evolve to become separate species. So, in a sense, this section on measuring the restriction of gene flow between hybridizing races has a direct impact on the discussion in the next section on speciation, since restricted gene flow sets the stage for reproductive isolation.

In the Poschiavo-Valtellina area, chromosomal races are distinguished by whole-arm rearrangements, with breakpoints at the centromeres (Figure 2); these are the regions of the genome where we should be expecting reduced gene flow between races, which may be reflected by greater genetic differentiation (Panithanarak et al. 2004). We have been examining genetic differentiation between two groups of races: CHPO and IMVA (group 1) vs. ILVA and IUVA (group 2) (Panithanarak et al. 2004; Giménez et al. 2013; Förster et al. 2016) that differ such that the F_1 hybrids produce a chain-of-five configuration (Figure 4), which is known to promote hybrid unfitness associated with germ cell death and malsegregation of chromosomes (Hauffe and Searle 1998) and recombination suppression (though not elimination) in the centromeric regions of the chain-of-five (Merico et al. 2013). Chromosomes 10 and 12 differ between the two groups of races (metacentric 8.12, and telocentrics 2, 10 in the first group and metacentrics 2.8, 10.12 in the second). Analysis of microsatellite markers along these two chromosomes showed that, as expected, the greatest differentiation was in the region of the centromeres (Panithanarak et al. 2004; Giménez et al. 2013). Simulation modelling suggests that differentiation could have resulted from hybrid unfitness on its own or a combination of hybrid unfitness and recombination suppression. A separate study showed that the centromeres of the other chromosomes of the chain-of-five (chromosomes 2 and 8) also showed differentiation between the hybridizing groups of races (Förster et al. 2016). Considering the chain-of-five (2-2.8-8.12-12.10-10; Figure 4), greater differentiation was seen for the internal chromosomes 8 and 12 than the external chromosomes 2 and 10. This may implicate recombination suppression, with a greater tendency in internal versus external for structural reasons (Förster et al. 2016).

Interestingly, differentiation was found in some genomic regions other than the centromeres of the chromosomes involved in the chain-of-five. This has not been studied systematically, but is known to involve some non-centromeric regions of the chain-of-five chromosomes (Giménez et al. 2013), and some centromeric regions from elsewhere (Förster et al. 2016). Epistasis is one possible explanation for this (Giménez et al. 2013; Förster et al. 2016). For example, if the genes in the centromeric regions of the chromosomes involved in the chain-of-five have race-specific alleles, those alleles may interact most favorably with particular alleles at loci that are unlinked or not closely linked, creating an epistatic relationship. This possibility and others need further exploration, building on the simulation modelling initiated in Giménez et al. (2013).

Therefore, our studies in the Poschiavo-Valtellina area indicate that gene flow is inhibited, particularly in the centromeric regions of the chromosomal rearrangements that differ between the hybridizing forms, where those rearrangements lead to formation of a chain-of-five configuration. Thus, if there are substantial chromosomal differences between hybridizing chromosomal races of house mouse such that a long chain configuration is produced, there are grounds for believing that that will be associated with a degree of interruption to gene flow near the breakpoints of the chromosomes that differ between the races. This could be important in speciation, including in the example of reproductive isolation in the Poschiavo-Valtellina area described in the next section.

These findings need to be put in the context of the considerable current interest in another type of chromosomal rearrangement which has a possible role in promoting genetic differentiation and reproductive isolation between hybridizing forms, namely chromosomal inversions (e.g. Neafsey et al. 2010; Joron et al. 2011; Stevison et al. 2011; Wadsworth et al. 2015; Lohse et al. 2015). The focus on this mutation has been on suppression of recombination within inversions and around inversion breakpoints (Rieseberg 2001; Ortiz-Barrientos et al. 2016). Centromeric regions (even when not associated with chromosomal rearrangements) also show low recombination and could be sites of accumulation of genetic differentiation (Neafsey et al. 2010; Nachman and Payseur 2012), and general models are being developed for the role of reduced recombination anywhere in the genome to have an impact on differentiation and speciation (Burri et al. 2015; Ortiz-Barrientos et al. 2016). What is interesting in the case of the house mouse system of chromosomal variation in general, and what we are observing in the Poschiavo-Valtellina area in particular, is that there is clearly demonstrable hybrid unfitness that may add to the impact of recombination suppression. All of these factors, namely a generalized lowering of recombination around the centromere, reduced recombination in the vicinity of the chromosomal breakpoint, and reduced gene flow in the vicinity of unfitness loci have an impact on the centromeric regions of rearranged chromosomes in hybridizing chromosomal races of house mice in the Poschiavo-Valtellina area.

Model of speciation

In the Poschiavo-Valtellina area, hybrids between certain chromosomal races (group 1 vs. group 2, see above) may produce a meiotic chain-of-five configuration that can cause unfitness (Hauffe and Searle 1998) and recombination suppression near the centromeres (Merico et al. 2013). This meiotic complex will have the most important role in potential speciation events. In terms of unfitness the emphasis on the hybrids with the chain-of-five follows very well-established models of 'chromosomal speciation': those of Capanna (1982) and Baker and Bickham (1986) which focused on the fitness disadvantages of hybrids between chromosomal races which produce long meiotic chains or rings (i.e. longer than the chain-of-three formed by heterozygotes for a single metacentric; see also King 1993, Searle 1993). It is only more recently that there has been an emphasis on recombination suppression near rearrangement breakpoints as a major role in speciation (Rieseberg 2001; Faria and Navarro 2010; see also Searle 1993). This is viewed as a very potent factor in speciation by some, e.g. Ortiz-Barrientos et al. (2016) write: "Evidence abounds that chromosomal rearrangements, via their suppression of recombination during meiosis in hybrids, play a major role in adaptation and speciation." Here, in the house mouse systems, we emphasize a potential role for both recombination suppression and unfitness in chromosomal speciation. Indeed the combination of recombination suppression and unfitness in jointly promoting speciation may not be limited to systems founded on whole-arm chromosomal rearrangements, as in the house mouse. Part of the interruption to gene flow associated with chromosomal inversions may also involve the production of chromosomally unbalanced products (and therefore unfitness) due to crossovers within the inversion (Ortiz-Barrientos et al. 2016). In the case of the house mouse, both recombination suppression and unfitness may reduce gene flow around the centromere and could potentially contribute to reproductive isolation in that way.

The interest in house mice from the Poschiavo-Valtellina area with regards chromosomal speciation relates to extraordinary data collected by Capanna and Corti (1982) and Mainardi et al. (1986). In the village of Migiondo (village 3; Figure 1), over a five-year period they found large numbers of CHPO and IUVA individuals without any hybrids (Table 2). The basis of this behavioral isolation is unknown, because by the time we started to study the village population, six years after the completion of the earlier study, one of the races had gone extinct (Hauffe and Searle 1992, 1993). Also, in two other nearby villages, we found that the same two races that failed to interbreed in Migiondo were able to produce hybrids (Table 2).

Combining our results and those of Capanna and Corti (1982) and Mainardi et al. (1986) suggested that there is something special about the Migiondo population that led to reproductive isolation there. The village is very isolated geographically and we showed, using an allozyme marker, that isolation and small population size may lead to genetic drift and fixation of rare variants (Fraguedakis-Tsolis et al. 1997). Given that the CHPO x IUVA hybrids are relatively unfit, because of the fertility problems associated with having a chain-of-five configuration (Hauffe and Searle 1998), then selection may have led to assortative mating (i.e. reinforcement: Servedio and Noor 2003). The reduced gene flow at the centromeric regions of the chromosomes in the chain-of-five configuration may have allowed an assortative mating locus to be in strong linkage disequilibrium with the chromosomal rearrangements defining the chromosome races (Piálek et al. 2001), in this way ensuring that no hybrids were produced in Migiondo. The isolation of the Migiondo population would have minimized the possibility of genetic swamping by alleles away from the contact area. Such incoming alleles would have reduced the chance of an absolute association of particular assortative mating alleles and a particular race.

It is notable that Migiondo is the only village in the Poschiavo-Valtellina area to show behavioral isolation between chromosomal races and that the behavioral isolation there was transient (in fact, very recently, hybrids were found in Migiondo suggesting interbreeding with IMVA, found only a few kilometers away in Vernuga: Figure 1; HC Hauffe and JB Searle unpublished data). These observations indicate that the attainment of reproductive isolation may sometimes be very local, and that the system of 'speciation' (the occurrence of two reproductively isolated forms together in the same place) may disappear after a few years (Hauffe and Searle 1992). Permanent reproductive isolation between chromosomal races of mice through reinforcement may therefore only happen very rarely after many false starts, which may not even be noticed. Even the term 'speciation' in this situation may be a misnomer. Although the CHPO and IUVA races in Migiondo were reproductively isolated from each other around 1980 (Table 2), they may not have been isolated from all other *M. m. domesticus* at that time. As with ring species, certain populations in contact may be unable to interbreed with each other, but retain the ability to interbreed with populations elsewhere in the species range.

Despite all these caveats, the description of behavioral isolation between chromosomal races of mice in the Poschiavo-Valtellina area is extremely interesting. Although the local 'speciation' event in the Poschiavo-Valtellina area did not lead to a new long-lasting species of house mouse, the fact that behavioral isolation has been seen to be associated with large-scale chromosomal difference gives credence to the idea that chromosomal rearrangements can be involved in speciation (in this case invoking whole-arm rearrangements). In different circumstances perhaps involving other species, the conditions that lead to behavioral isolation may actually allow two chromosomal races to become completely reproductively isolated from each other.

Because the house mouse is dependent on human-created habitat over much of its range, especially in the Alps, and because of the unusual mixing and bottlenecking of populations that arise from that anthrodependence, coupled with an intrinsic high rate of chromosomal mutation (Nachman and Searle 1995), the opportunity is considerable for chromosomal rearrangements to lead to 'speciation' events. To further illustrate that humans can be important in generating opportunities for 'speciation' events, the ragwort *Senecio* is an instructive parallel to what we describe for the Poschiavo-Valtellina house mice (Abbott et al. 2009). In this case the new stable diploid hybrid species *S. squalidus* originated from the human introduction of individuals from an Italian *Senecio* hybrid zone to a botanic garden in Britain 300 years ago; this new hybrid form then spread and hybridized with a tetraploid native *Senecio* generating a new tetraploid hybrid species (*S. eboracensis*) and an allohexaploid species (*S. cambrensis*). Humans were involved in inadvertently generating *S. squalidus* by transporting plants across Europe, and they aided its spread in Britain by creating appropriate habitat for the species (the substrate standardly used for railway lines). There is a chromosomal element to the generation of *S. cambrensis* (polyploidy in this case) and the transient nature of some human-mediated 'speciation' events is illustrated by the fact that *S. eboracensis* went extinct only 20 years after its discovery. Given the ability of the *Senecio* 'species' to successfully hybridize with each other, as with the case in the house mouse, it may be inappropriate to consider the generation of these forms as true 'speciation'. However, we would argue that Migiondo mice and the British ragworts are providing aspects of the varied perspective that is needed to understand the speciation process.

Conclusions

The Poschiavo-Valtellina area is relatively small, but there is a remarkable patchwork of chromosomal races of house mouse which have provided a testbed for a variety of studies relating to the origin of the races and the impact of their interaction. The house mouse is the classic mammalian evolutionary model system, with the ability to breed individuals (to examine fertility etc.) and to have had access to genomic tools (to examine the impacts of hybridization for specific regions of the genome etc.). These advantages continue for the future investigation of the mice in the Poschiavo-Valtellina area, and to make further progress in our understanding of karyotypic diversification and the role of chromosomal rearrangements in speciation.

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Table 1. The chromosomal races found in the Poschiavo-Valtellina area including abbreviated race name (Piálek et al. 2005) and the disposition of chromosomes as telocentrics (1, 2 etc.) or metacentrics (1.3, 2.8 etc.) with race-defining metacentrics in bold

Standard	ST40	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	XX/XY
Poschiavo	CHPO	1.3	2		4.6	5.15		7	**8.12**	9.14	10	11.13					16.17		18	19	XX/XY
Mid Valtellina	IMVA	1.3	2		4.6	5.15		**7.18**	**8.12**	9.14	10	11.13					16.17			19	XX/XY
Upper Valtellina	IUVA	1.3	**2.8**		4.6	5.15		7		9.14	**10.12**	11.13					16.17		18	19	XX/XY
Lower Valtellina	ILVA	1.3	**2.8**		4.6	5.15		**7.18**		9.14	**10.12**	11.13					16.17			19	XX/XY

Table 2. The frequency of CHPO and IUVA in villages where both races are present based on data from Capanna and Corti (1982), Mainardi et al. (1986) and Hauffe and Searle (1992, 1993)

	CHPO	**CHPO x IUVA F_1 hybrid**	**IUVA**	**Other karyotypes**
Migiondo 1978-1983 (3)	**90**	**0**	**60**	
Migiondo 1989-1990 (3)	**37**	**0**	**0**	
Sommacologna 1990-1991 (2)	**3**	**2**	**3**	**1**
Sondalo 1990-1991 (1)	**2**	**2**	**5**	**4**

The villages are mapped in Figure 1, and the numbers in brackets match with the village ID in that figure.

Figure 1 Map of the Poschiavo-Valtellina area at the alpine border between Italy and Switzerland, showing the distributions of the standard race (ST40) and four metacentric races (CHPO, IMVA, IUVA, ILVA), and their hybrids. Grey shaded areas: villages. Colored shaded areas: distributions previously published by other authors (ILVA: Capanna and Valle 1977; Gropp et al. 1982; Capanna et al. 1985; CHPO: Gropp et al., 1970, 1972). Villages are usually dominated by a single pure chromosomal race (i.e in its fully homozygous state), but some chromosomally-identifiable hybrids with another race are often present; sometimes other situations are found (e.g. two pure races and hybrids). Thus, [race]+hyb[race] indicates the pure race and hybrids with another named race; [race]x[race] means that two pure races and F_1 hybrids have been found. There are no pure ST40 villages. The villages sampled by us between 1989 and present are: 1. Sondalo; 2. Sommacologna; 3. Migiondo (note: IUVA was also found here in 1982 but no hybrids; we have unpublished recent evidence of hybridization of CHPO with IMVA); 4. Vernuga; 5. Grosio; 6. Grosotto; 7. Prada; 8. Vione; 9. Vervio; 10 Nova; 11. Tovo di S. Agata; 12. Mazzo; 13. Sontiolo; 14. Tiolo; 15. Lago; 16. Lovero; 17. Sernio; 18. Biolo; 19. Tirano; 20. Villa di Tirano; 21. Tresenda; 22. S. Giacomo; 23. Polaggia; 24. Berbenno. Angeli Custodi (mentioned in the text) is the northernmost village under the blue shaded area in Val Poschiavo. This map was created using Microsoft Encarta Premium Suite (2003).

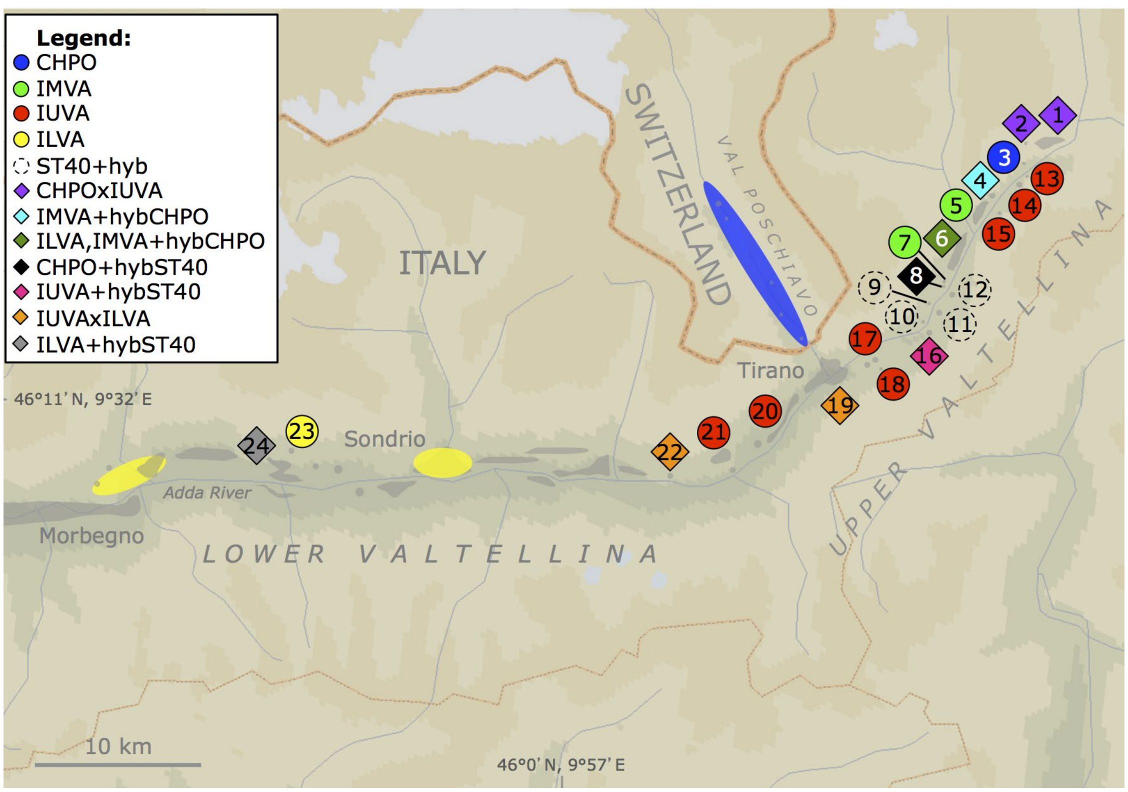

Figure 2. Whole-arm chromosomal rearrangements as illustrated with diagrammatic mouse chromosomes. A) Robertsonian (Rb) fusion of two telocentric chromosomes to generate a metacentric. B) Rb fission of a metacentric to generate two telocentrics. C) Whole-arm reciprocal translocation (WART) involving two metacentric chromosomes. D) WART between a metacentric and a telocentric chromosome. These mutations all involve chromosome breakage in the centromeric region. Telocentric chromosomes have substantial quantities of minor satellite sequence that have a role in centromere organization. This minor satellite DNA is largely lost on Rb fusion (and the telomeric sequence is completely lost), presumably reducing the chances of Rb fission, which requires the generation of two telocentrics with functioning centromeres and telomeres (Garagna et al. 1995, 2001).

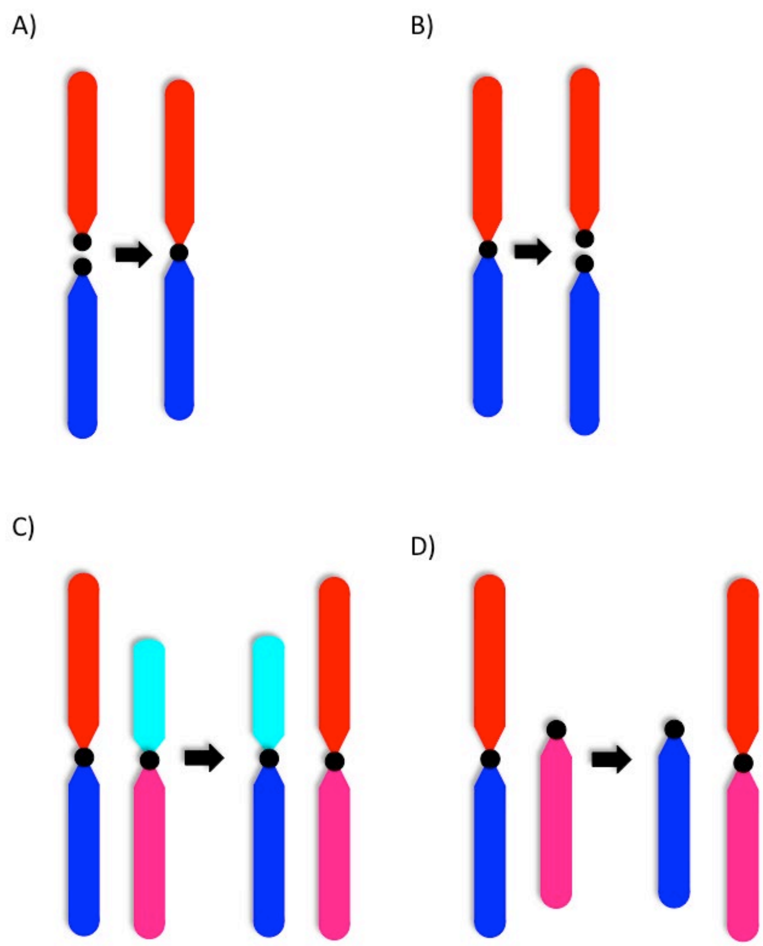

Figure 3. G-band karyotypes of A) an ILVA female mouse, B) an ILVA x IUVA F_1 hybrid male mouse and C) an IUVA female mouse, the three karyotypes found in San Giacomo village (see text)

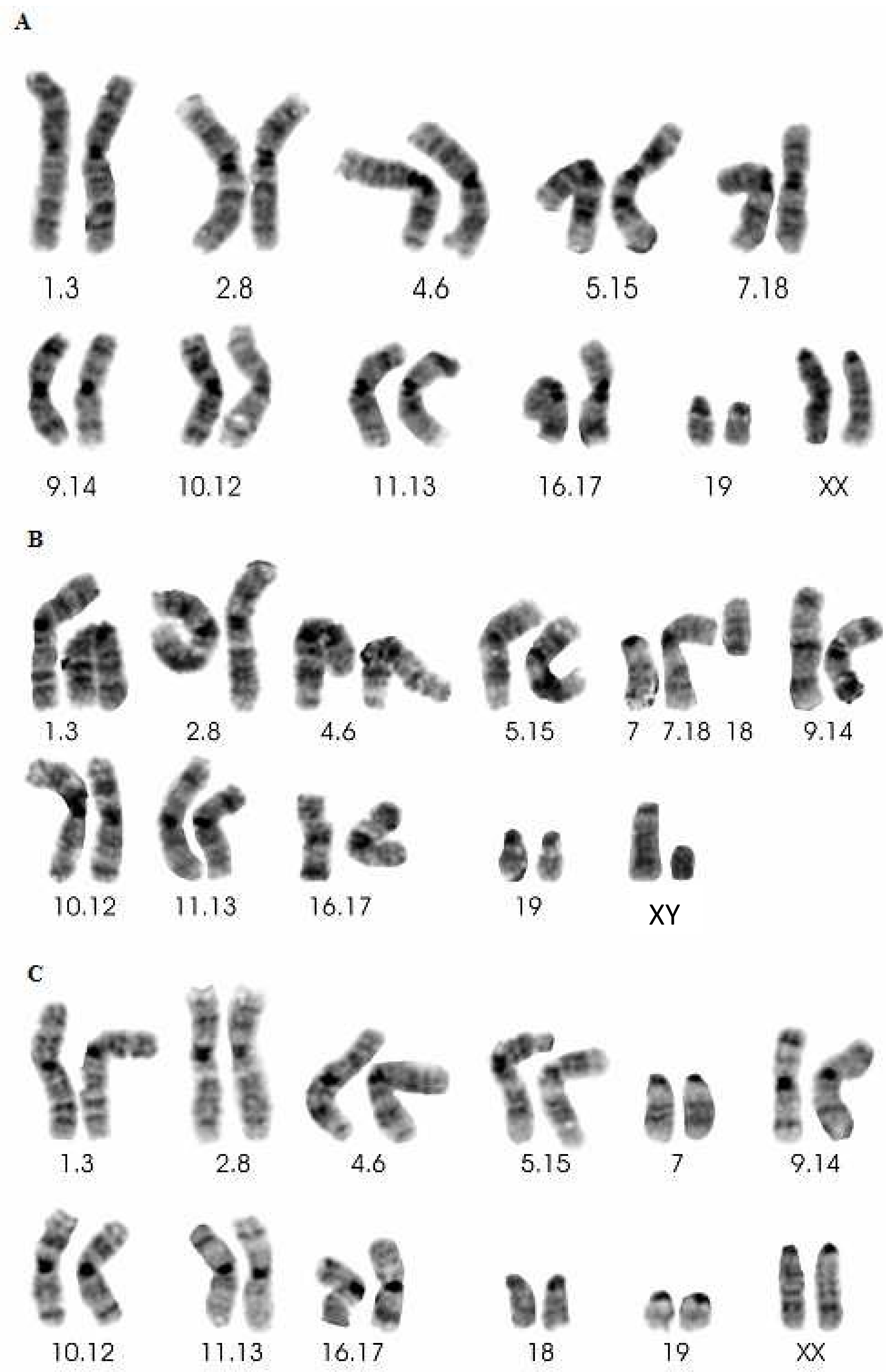

Figure 4. Diagrams of anaphase I multivalent configurations (a chain-of-five and a chain-of-three) of the F_1 hybrid between CHPO and ILVA.